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Four new species of *Anenterotrema* Stunkard, 1938 (Digenea: Dicrocoellidae) infecting phyllostomid bats in the Peruvian Amazon

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Abstract During a research of digeneans (Platyhelminthes: Trematoda) infecting leaf-nosed bats (Chiroptera: Phyllostomidae) from the Kawsay Biological Station in Madre de Dios, southeastern Peru, four new species were described based on light and scanning electron microscopy (SEM): *Anenterotrema paramegacetabulum* **n. sp.** from the seba's short-tailed bat *Carollia perspicillata* Linnaeus, *A. hastati* **n. sp.**, *A. kawsayense* **n. sp.**, and *A. peruense* **n. sp.** from the greater spear-nosed bat *Phyllostomus hastatus* (Pallas). *Anenterotrema paramegacetabulum* **n. sp.** differs from all congeners by having a terminal oral sucker, a transversely elongated ventral sucker without clamp-shaped, and by having the testes located immediately posterior to ventral sucker. *Anenterotrema hastati* **n. sp.** is easily differentiated from the other congeneric species by having an almost clamp-shaped oral sucker, well-developed cirrus sac, a bilobulated seminal receptacle, and a group of well-developed unicellular glands located anterolateral to cirrus sac. *Anenterotrema kawsayense* **n. sp.** is characterized by possessing protuberances on the anterior margin of the oral sucker. *Anenterotrema peruense* **n. sp.** is mainly characterized by having the testes located principally anterior to ventral sucker and the cirrus sac located perpendicular to midline of the body. The present finding brings to 12, the number of known species of *Anenterotrema*. A key to the species of *Anenterotrema* Stunkard, 1938 is provided.

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Introduction

Leaf-nosed bats (Chiroptera: Phyllostomidae) due to their diversity, distribution and, in certain species, their adaptability to inhabit disturbed places, make them a good host model for harboring diverse groups of organisms as viruses, bacteria, fungus, and helminths (Calle & Arango, 1999; Murillo-García & Bedoya-Durán, 2014; Adhikari et al., 2020). There are about 1386 species of bats worldwide (Burgin et al., 2018). In South America, 302 (21%) species belonging to nine families and 80 genera are known (Díaz et al., 2016). Despite this diversity, only 92 species (30%) have been studied from a parasitological approach, with about 119 helminth parasite species recorded (Santos & Gibson, 2015; Achatz et al., 2018; Fernandes et al., 2019; Simões et al., 2019; Fernandes et al., 2021a,b). One hundred eighty-nine species of bats have been registered or described in Peru (Pacheco et al., 2021), but few studies on their parasitic helminths were performed (Minaya et al., 2020), remaining practically unexplored, especially in the Madre de Dios region, a region with a high biological potential. In this region, barely two species of helminth parasites of bats are known, namely *Litomosoides chandleri* Esslinger, 1973 from *Artibeus planirostris* Spix and *L. solarii* Guerrero, Martin, Gardner & Bain, 2002 infecting *Trachops cirrhosus* Spix (Guerrero et al., 2002; Santos & Gibson, 2015; Minaya et al., 2020).

During a parasitological survey of Neotropical bats, several digeneans specimens were recovered from the intestines of two leaf-nosed bats from the Kawsay Biological Station in Madre de Dios region (Peruvian Amazon). Detailed morphological examination based on light and scanning electron microscopy (SEM) revealed that the digeneans represent four new species of *Anenterotrema* Stunkard, 1938 (Trematoda: Dicrocoellidae), which are described and illustrated herein. In addition, Lunaschi & Drago's (2011) key to the species of *Anenterotrema* is revised to include the four new species.

Key to the species of *Anenterotrema* Stunkard, 1938 (modified from Lunaschi & Drago, 2011)

1. Oral sucker subterminal ……. 2
   Oral sucker terminal ……..…… *Anenterotrema paramegacetabulum* **n. sp.**
2. Oral sucker with lateral and dorsal collar-like tegumental thickening……..…. 3
   Oral sucker without lateral and dorsal collar-like tegumental thickening ……..... 5
3. Testes large in relation to body size ...……...…… *Anenterotrema auriatum*
   Testes small to medium in relation to body size …...……...……...……..... 4
4. Cirrus sac 42–61 × 37–43 …….. *Anenterotrema freitasi*
   Cirrus sac 82–83 × 60–64 ……...… …...…… *Anenterotrema eduardocaballeroi*
5. Testes anterior to ventral sucker …..... .....……. *Anenterotrema peruense* **n. sp.**
   Testes at level or posterior to ventral sucker ……..……..... 6
6. Ovary close to posterior end of body..... *Anenterotrema stunkardi*
   Ovary not close to posterior end of body…….……..……..... 7
7. Ratio to ventral sucker width:body width < 0.6 ……..... 8
   Ratio to ventral sucker width:body width >0.6...…..... *Anenterotrema megacetabulum*
8. Suckers with variabel aperture...…… 9
   Suckers with close aperture, reduced to longitudinal slit.....…..… *Anenterotrema mesolecitha*
9. With a group of unicellular glands located anterolateral to cirrus sac ……..……..……10
   Without a group of unicellular glands located anterolateral to the cirrus sac……..……..… 11
10. Body shape elliptoid and oral sucker broadly elliptoid……..……...… *Anenterotrema kawsayense* **n. sp.**
    Body shape pyriform and oral sucker almost clamp-shaped……..……. *Anenterotrema hastati* **n. sp.**
11. Ratio oral:ventral sucker width 0.86–1.16 and cirrus sac length 39–79……. *Anenterotrema liliputianum*
    Ratio oral:ventral sucker width 0.6–0.8 and cirrus sac length 114–130 …... *Anenterotrema iannaconei*

Materials and methods

For 12 days of sampling between July to August 2021, 10 phyllostomid bats, 6 *Carollia perspicillata* (Linnaeus) and 4 *Phyllostomus hastatus* (Pallas), were collected by mist-netting from the Kawsay Biological Station in Madre de Dios (12°31'S, 69°0'W), Peru. The nets were opened from 17:30 to 23:00 hours, checked every 30 minutes. The hosts were handled, identified in situ by the specialist and euthanized following Sikes (2016). The bat collection was authorized by the wildlife department in Peru with Directorial Resolution N°D000037-2021-MIDAGRI-SERFOR-DGGSPFFS-DGSPFS. For parasitological examination, guts were immediately removed and placed in vials containing heated ethanol solution 70% (70°C). In the laboratory, the contents of each vial were examined under a dissecting microscope and digeneans were removed from the intestines using small probes. Digenean specimens used for light microscopy were stained with hydrochloric carmine or Mayer's Paracarmine, dehydrated in an ethanol series of ascending concentrations, clarified in Eugenol (4-allyl-2-ethoxyphenol), and mounted in Canada balsam. Specimens were examined and photographed using a compound Nikon™ Eclipse SI photomicroscope equipped with phase contrast microscopy optics and drawings were made with the aid of a drawing tube. Measurements are in micrometers, unless otherwise indicated, using straight-line distances between extreme points of the structures measured and are expressed as the range followed by the mean and number (n) of structures measured in parentheses. Specimens observed under a scanning electron microscopy (SEM) were dehydrated through a graded ethanol series, critical point dried with carbon dioxide, coated with gold and examined in an Inspect S50 - FEI, at an accelerating voltage of 10 kV (Huancachoque et al., 2020). Type material was deposited in the Helminthological Collection of the Museum of Natural History at the San Marcos University (MUSM-HEL), Lima, Peru.

To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of new taxon has been submitted to ZooBank. The Life Science Identifier (LSID) is reported in the taxonomic summary.

Order Plagiorchiida
Dicrocoeliidae Loss, 1899
***Anenterotrema* Stunkard, 1938**
***Anenterotrema paramegacetabulum* n. sp. Cacique, Cruces & Chero**

Type-host: *Carollia perspicillata* (Linnaeus) (Chiroptera: Phyllostomidae), seba's short-tailed bat.
Site in host: small intestine.
Prevalence: Four of six hosts infected (67%) with a total 62 worms.
Mean Intensity: 16 digeneans per infected host (range 9–20).
Type-locality: Kawsay Biological Station (12°31'44''S, 69°0'26''W), Madre de Dios, Peru, South America.
Type-material: Holotype (MUSM-HEL 5120); 14 paratypes (MUSM-HEL 5121a-n).
ZooBank registration: The Life Science Identifier (LSID) for *Anenterotrema paramegacetabulum* **n. sp.** is urn:lsid:zoobank.org:act:C9734C90-C77E-45DC-82AB-331D82607D59.
Etymology. The specific name refers to the resemblance of the new species to *Anenterotrema megacetabulum* Fernandes, Santos, Melo, Achatz & Tkach, 2021.

Description (Fig. 1A, Fig. 2A-F)

[Based on 15 adult specimens.] Body broadly pyriform, 266–446 (392; n = 15) long; greatest width, 203–371(328; n = 15) at level posterior of ventral sucker. Body width:length ratio 0.73–0.93 (0.84; n = 15). Forebody length 90–160 (132, n=15). Forebody: body length ratio 0.30–0.39 (0.34 = 15). Tegument smooth. Oral sucker terminal, oval, 62–100 × 90–127 (82 × 117; n = 15) wide, with three papillae at each side of oral sucker. Oral sucker length: body length 0.17–0.26 (0.21; n = 15). Ventral sucker transversely elongated, lacking aperture, strongly protruding above ventral surface, located slightly anterior to midbody, surrounded by randomly arranged papillae, 91–108 × 127–205 (100 × 176; n = 15). Ventral sucker width: body width ratio 0.49–0.63 (0.53; n = 15). Oral sucker

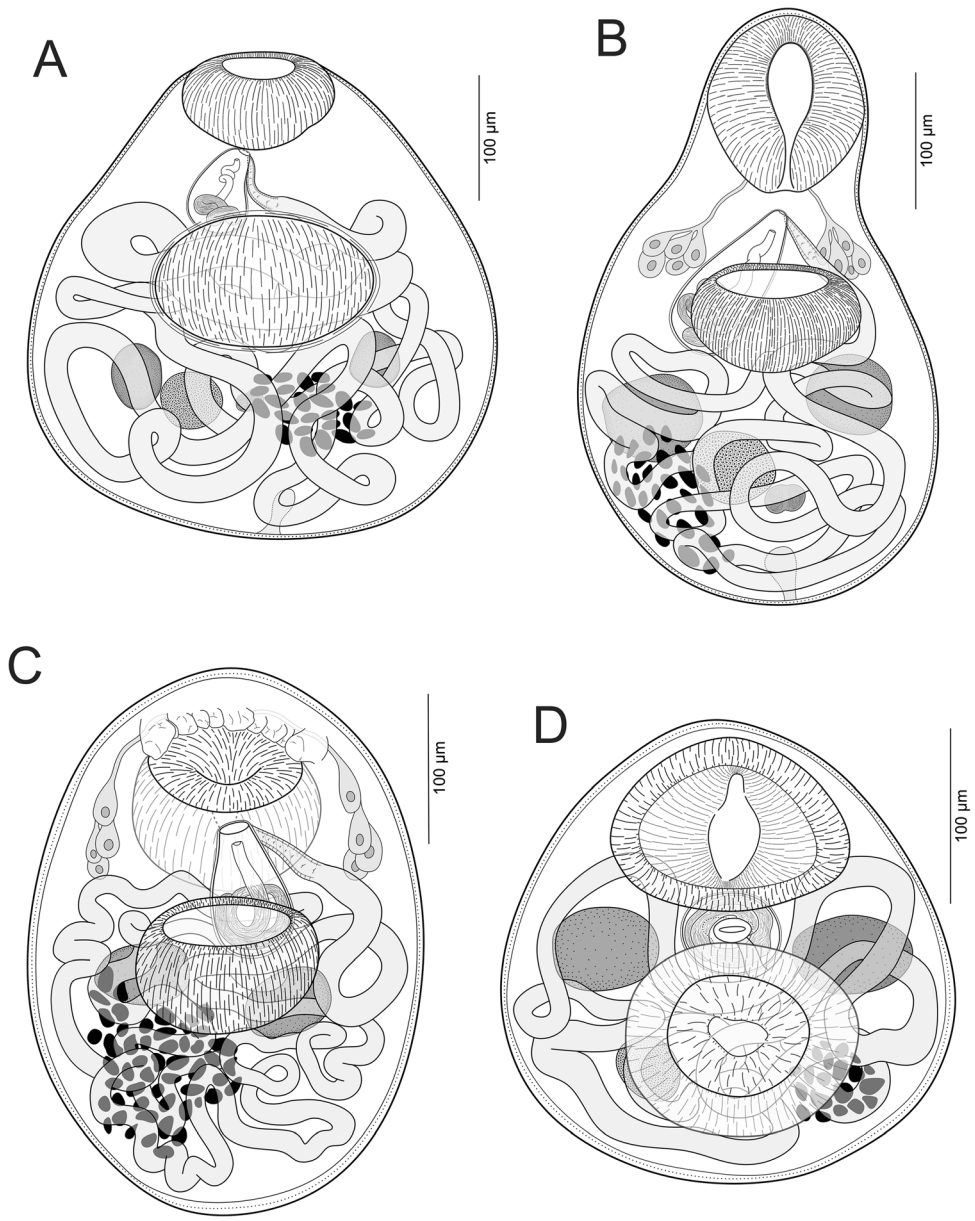

Fig. 1 *Anenterotrema* spp. infecting leaf-nosed bats from Madre de Dios region in Peru, whole worms, ventral view. A, *Anenterotrema paramegacetabulum* **n. sp.** ex *Carollia perspicillata*; B, *Anenterotrema hastati* **n. sp.** ex *Phyllostomus hastatus*; C, *Anenterotrema kawsayense* **n. sp.** ex *Phyllostomus hastatus*; D, *Anenterotrema peruense* **n. sp.** ex *Phyllostomus hastatus*.

width:ventral sucker width ratio 0.60–0.73 (0.66; n =15). Pharynx, esophagus and intestinal caeca absent. Testes opposite, slightly diagonal, oval, principally post-equatorial, located immediately posterior to ventral sucker, sometimes slightly overlapping with ventral sucker, right testis 37–67 × 34–64 (54 × 48; n = 9), left testis 40–65 × 41–83 (53 × 57; n = 6). Cirrus sac oval, thin-walled, posteriorly overlapping with anterior margin of ventral sucker, 57–89 × 41–59 (78 × 50; n = 14). Cirrus sac containing coiled seminal vesicle and unarmed, muscular cirrus. Prostatic glands not observed. Genital pore sub-median, between oral and ventral suckers. Ovary 43–59 × 42–50 (51 × 46; n = 4), inter-testicular, sometimes situated slightly posterior and dorsal to testes. Seminal receptacle small, sinistral to ovary, strongly obscured by uterus.

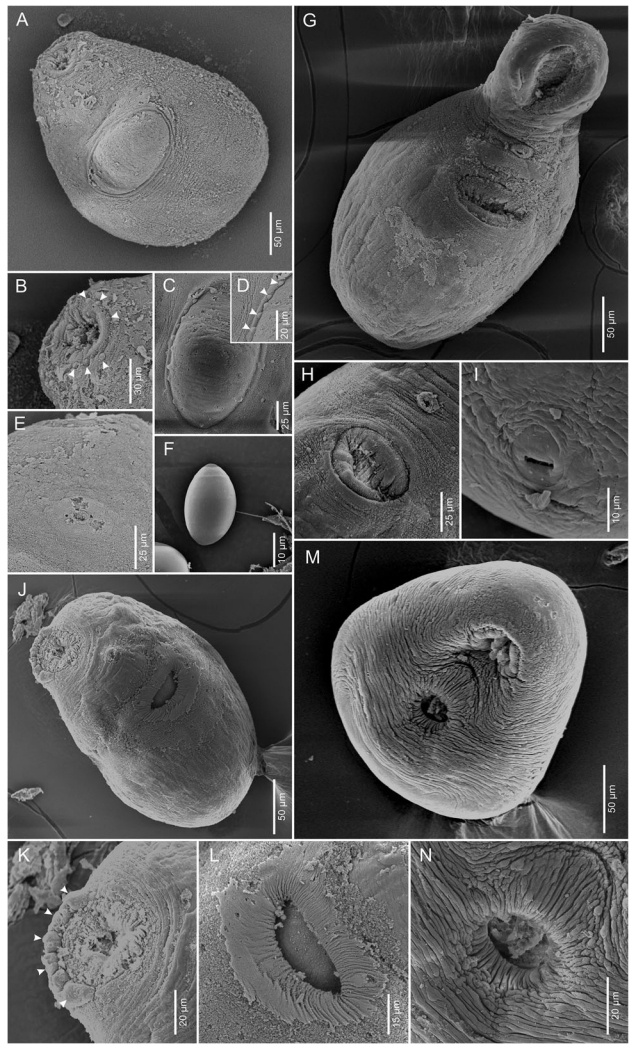

Fig. 2 Scanning eléctron micropraphs of *Anenterotrema* spp. A, *Anenterotrema paramegacetabulum* n. sp., ventral view; B, Oral sucker with papillae (white arrow); C, Ventral sucker; D, Papillae on entral sucker (white arrow); E, Genital pore; F, Egg; G, *Anenterotrema hastati* **n. sp.**, ventral view; H, Genital pore and ventral sucker; I, Excretory pore; J, *Anenterotrema kawsayense* **n. sp.**, ventrolateral view; K, Oral sucker with protuberances on its anterior margin (white arrow); L, Ventral sucker; M, *Anenterotrema peruense* **n. sp.**, ventral view; N, Ventral sucker.

Mehlis' gland and Laurer's canal not observed. Vitellarium consists of numerous small follicles, dextral or sinistral in hinbody, principaly obscured by uterus. Uterus thin-walled, strongly developed, occupying entire hindbody, rarely extending slightly anterior to forebody, typically overlapping gonads. Metraterm well-developed, thick-walled, strongly muscular. Eggs operculate, 33–37 × 19–24 (35 × 21; n = 14). Body length:egg length ratio 10.33–13.39 (11.51; n = 15). Excretory pore terminal; excretory vesicle small, saccate.

Anenterotrema paramegacetabulum **n. sp.** is the only *Anenterotrema* species having a terminal oral sucker. The new species is most similar to *Anenterotrema megacetabulum* Fernandes, Santos, Melo, Achatz & Tkach, 2021 from *Molossus molossus* (Pallas) in Brazil by having a well-developed and transversely elongated ventral sucker. However, the new species differs from *A. megacetabulum* by having a ventral sucker without clamp-shaped (with clamp-shaped in *A. megacetabulum*), lacking aperture (longitudinal aperture in *A. megacetabulum*), and by the

position of the testes (immediately posterior to ventral sucker in the new species vs overlapping with ventral sucker in *A. megacetabulum*). In addition, the new species can be easily distinguished from *A. megacetabulum* by having lower forebody:body length ratio values (0.30–0.39 in *A. paramegacetabulum* **n. sp.** vs 0.46–0.57 in *A. megacetabulum*), higher body width:-body length ratio values (0.73–0.93 in the new species vs 0.37–0.48 in *A. megacetabulum*), and by the size of the eggs (33–37 in *A. paramegacetabulum* **n. sp.** vs 24–27 in *A. megacetabulum*) (Table 1).

***Anenterotrema hastati* n. sp. Cacique, Cruces & Chero**

Type-host: *Phyllostomus hastatus* (Pallas) (Chiroptera: Phyllostomidae), greater spear-nosed bat.

Site in host: duodenum.

Prevalence: Three of four hosts infected (75%) with a total 42 worms.

Mean Intensity: 14 digeneans per infected host (range 7–16).

Co-infection: This species was found infecting *P. hastatus* along with *A. kawsayense* **n. sp.** and *A. peruense* **n. sp**.

Type-locality: Kawsay Biological Station (12°31'44''S, 69°0'26''W), Madre de Dios, Peru, South America.

Type-material: Holotype (MUSM-HEL 5122); 11 paratypes (MUSM-HEL 5123a-k).

ZooBank registration: The Life Science Identifier (LSID) for *Anenterotrema hastati* **n. sp.** is urn:lsid:-zoobank.org:act:CEC7E77C-F0E6-4406-8C92-EBA9B137BC0E.

Etymology. The specific name of the new species refers to the type-host.

Description (Fig. 1B, Fig. 2G-I)

[Based on 12 adult specimens]. Body pyriform, 421–591 (485; n = 12) long; greatest width, 206–296 (264; n = 12) at level of testes. Body width:length ratio 0.49–0.61 (0.55; n = 12). Forebody length 161–268 (203, n=12). Forebody: body length ratio 0.37–0.46 (0.42 = 12). Tegument smooth. Oral sucker subterminal, subspherical, large, almost clamp-shaped, 100–138 × 107–129 (124 × 120; n = 12). Oral sucker length: body length 0.23–0.29 (0.26; n = 12). Ventral sucker oval, located anterior to middle third of body, 92–119 × 98–131 (105 × 119; n = 12). Ventral sucker width: body width ratio 0.42–0.49 (0.45; n = 12). Oral sucker width:ventral sucker width ratio 0.84–1.13 (1.01; n = 12). Pharynx, esophagus and intestinal caeca absent. Group of well-developed unicellular glands located anterolateral to cirrus sac. Testes opposite, slightly diagonal, oval, principally post-equatorial, located immediately posterior at ventral sucker; right testis 36–64 × 30–54 (48 × 48; n = 6); left testis 34–63 × 38–72 (51 × 55; n = 8). Cirrus sac pyriform, well-developed, thin-walled, frequently reaches the middle part of ventral sucker or surpasses ventral sucker, 93–150 × 41–82 (119 × 61; n = 11). Cirrus sac with long coiled seminal vesicle; cirrus muscular, unarmed. Prostatic glands not observed. Genital pore median, between oral and ventral suckers. Ovary 43–59 × 42–50 (51 × 46; n = 4), inter-testicular, situated near to one testes. Seminal receptacle small, bilobulated, sinistral to ovary, strongly obscured by uterus. Mehlis' gland and Laurer's canal not observed. Vitellarium consists of numerous small follicles, dextral or sinistral in hinbody, principaly obscured by uterus. Uterus thin-walled, strongly developed, occupying entire hindbody, typically overlapping gonads. Metraterm well-developed, thick-walled, strongly muscular (Fig. 1B). Eggs operculate, 25–32 × 17–19 (29 × 18; n = 12). Body length:egg length ratio 15.40–18.47 (16.58; n = 12). Excretory pore terminal; excretory vesicle small, saccate.

Remarks

Anenterotrema hastati **n. sp.** could be distinguished from all congeners by having an almost clamp-shaped oral sucker, well-developed cirrus sac, a bilobulated seminal receptacle, and a group of well-developed unicellular glands located anterolateral to cirrus sac. The new species slightly resembles *A. stunkardi* Caballero & Grocott, 1959 and *A. megacetabulum* by having almost similar oral sucker shape. However, *A. hastati* **n. sp.** differs from *A. stunkardi* by the position of the testes (located immediately posterior at ventral sucker in the new species vs at level of ventral sucker in *A. stunkardi*) and by the position of the ovary (situated near to one of the testes in *A. hastati* **n. sp.** vs well separated from testes in *A. stunkardi*). *Anenterotrema hastati* **n. sp.** can be differentiated from *A. megacetabulum* by the ventral sucker shape (oval in *A. hastati* **n. sp.** vs usually transversely elongated in *A. megacetabulum*) and by position of the ovary

Table 1 Morphometric comparison of the new *Anenterotrema* species and the most similar species (*Anenterotrema megacetabulum*, *A. liliputianum*, and *A. stunkardi*). Range values are followed by mean values in parentheses.

Species	*A. paramegacetabulum* **n. sp.**	*A. hastati* **n. sp.**	*A. kawsayense* **n. sp.**	*A. peruense* **n. sp.**	*A. megacetabulum*	*A. liliputianum*	*A. stunkardi*
Host	*Carollia perspicillata*	*Phyllostomus hastatus*	*P. hastatus*	*P. hastatus*	*Mollosus molossus*	*Molossidae* sp.	*P. hastatus panamensis*
Locality	Peru	Peru	Peru	Peru	Brazil	Brazil	Panamá
Reference	Present study	Present study	Present study	Present study	Fernandes et al., 2021	Lunaschi & Drago, 2011	Lunaschi & Drago, 2011
Number of Specimens	*n* = 15	*n* = 12	*n* = 8	*n* = 7	*n* = 12	*n* = 4	*n* = 10
Body length	266–446 (392)	421–591(485)	356–432 (387)	262–310 (281)	352–496 (450)	275–362 (306)	580–706 (649)
Body width	203–371 (328)	206–296 (264)	225–374(245)	240–293(265)	142–235 (186)	184–251(205)	290–396 (356)
Forebody length	90–160 (132)	161–268 (203)	121–172 (145)	106–142 (124)	142–279 (222)	105–145 (121)	227–304 (252)
Forebody:body length	0.30–0.39 (0.34)	0.37–0.46 (0.42)	0.33–0.42 (0.37)	0.39–0.48 (0.44)	0.46–0.57 (0.49)	0.58–0.6* (0.59)	0.41–0.42**(0.42)
Body width:body length	0.73–0.93 (0.84)	0.49–0.61 (0.55)	0.55–0.72 (0.63)	0.84–1.04 (0.94)	0.37–0.48 (0.41)	0.66–0.74* (0.7)	0.54–0.6**(0.57)
Oral sucker length	62–100 (82)	100–138 (124)	109–127 (118)	75–120 (95)	85–128 (112)	80–107 (90)	119–148 (125)
Oral sucker width	90–127 (117)	107–129(120)	104–132(120)	128–145(137)	65–100 (90)	93–113(103)	107–148(120)
Oral sucker lenght:body length	0.17–0.26 (0.21)	0.23–0.29 (0.26)	0.27–0.33 (0.30)	0.27–0.39 (0.34)	0.2–0.3 (0.3)	0.30–0.38* (0.34)	0.18–0.21**(0.20)
Ventral sucker length	91–108 (100)	92–119 (105)	96–110 (101)	93–127 (114)	87–132 (115)	89–107 (99)	97–131 (113)
Ventral suker width	127–205 (176)	98–131 (119)	113–132 (119)	127–146 (134)	127–182 (150)	107–121 (113)	100–155 (122)
Ventral sucker width:body width	0.49–0.63 (0.53)	0.42–0.49 (0.45)	0.46–0.56 (0.49)	0.47–0.57 (0.51)	0.6–1 (0.8)	0.44–0.54* (0.49)	0.30–0.36**(0.33)
Oral:ventral sucker width	0.60–0.73 (0.66)	0.84–1.13 (1.01)	0.91–1.13 (1.00)	0.97–1.10 (1.02)	0.5–0.8 (0.6)	0.8–1 (0.9)	0.8–1.2
Left testis length	40–65 (53)	34–63 (51)	33–60 (50)	37–39 (38)	42–65 (54)	58–66 (62)	64–130 (89)
Left testis width	41–83 (57)	38–72 (55)	34–60(45)	42–59 (52)	40–59 (54)	48–56 (52)	69–135 (86)
Right testis length	37–67 (54)	36–64 (48)	48–52 (51)	34–43 (38)	53–60 (56)	58–60 (59)	59–126 (81)
Right testis width	34–64 (48)	30–54 (48)	41–49 (44)	48–62 (52)	47–62 (57)	53–73 (63)	62–135 (82)
Cirrus sac length	57–89 (78)	93–150 (119)	77–113 (94)	-	62–108 (87)	39–79 (53)	63–102 (79)
Cirrus sac width	41–59 (50)	41–82 (61)	40–60 (50)	54–78 (63)	40–63 (55)	54–58 (57)	76–145 (109)
Ovary length	43–59 (51)	43–59 (51)	-	33–47(40)	43–68 (54)	34–37 (36)	71–100 (80)
Ovary width	42–50 (46)	42–50 (46)	-	38–52 (47)	47–52 (50)	31–64 (48)	71–95 (83)
Egg length	33–37 (35)	25–32 (29)	28–31 (29)	27–31 (29)	24–27 (26)	27–32 (29)	26–30 (29)

Table 1 continued

Species	*A. paramegacetabulum* **n. sp.**	*A. hastati* **n. sp.**	*A. kawsayense* **n. sp.**	*A. peruense* **n. sp.**	*A. megacetabulum*	*A. liliputianum*	*A. stunkardi*
Egg width	19–24 (21)	17–19 (18)	17–20 (19)	18–20 (18)	13–16 (14)	17–22 (20)	17–20 (18)
Body:egg length	10.33–13.39 (11.51)	15.40–18.47 (16.58)	12.28–13.94 (13.31)	9.03–11.07 (9.82)	13.0–19.8 (17.4)	9–13 (10)	20–25

*Values according to Fernandes et al. (2021).

**Values not provided in the published description, but inferred from the line drawing provided by Lunaschi & Drago (2011).

(posterior to ventral sucker in the new species vs partially overlapping with ventral sucker in *A. megacetabulum*).

***Anenterotrema kawsayense* n. sp. Cacique, Cruces & Chero**

Type-host: *Phyllostomus hastatus* (Pallas) (Chiroptera: Phyllostomidae), greater spear-nosed bat.

Site in host: duodenum.

Prevalence: Three of four hosts infected (75%) with a total 20 worms.

Mean Intensity: 5 digeneans per infected host (range 2–8).

Co-infection: This species was found infecting *P. hastatus* along with *A. hastati* **n. sp.**

Type-locality: Kawsay Biological Station (12°31'44''S, 69°0'26''W), Madre de Dios, Peru, South America.

Type-material: Holotype (MUSM-HEL 5124); 7 paratypes (MUSM-HEL 5125a-g).

Site in host: duodenum.

ZooBank registration: The Life Science Identifier (LSID) for *Anenterotrema kawsayense* **n. sp.** is urn:lsid:zoobank.org:act:FA36EBB2-5607-4C14-8ABB-D2B2D1B884B5.

Etymology. The specific name refers to the type locality.

Description (Fig. 1C, Figs. 2J-L)

Based on 8 adult specimens. Body elliptoid, 356–432 (387; n = 8) long; greatest width, 225–374(245; n = 8) at level of posterior ventral sucker. Body width:length ratio 0.55–0.72 (0.63; n = 8). Forebody length 121–172 (145, n=8). Forebody: body length ratio 0.33–0.42 (0.37 = 8). Tegument smooth. Oral sucker subterminal, very broadly elliptoid, 109–127 × 104–132 (118 × 120; n = 8). Conspicuous protuberances on anterior margin of oral sucker. Oral sucker length: body length 0.27–0.33 (0.30; n = 8). Ventral sucker oval, located principally in middle third of body, 96–110 × 113–132 (101 × 119; n = 8). Ventral sucker width: body width ratio 0.46–0.56 (0.49; n = 8). Oral sucker width:ventral sucker width ratio 0.91–1.13 (1.00; n =8). Pharynx, esophagus and intestinal caeca absent. Group of well-developed unicellular glands located anterolateral to cirrus sac. Testes opposite, oval, principally post-equatorial, overlapping with ventral sucker, right testis 48–52 × 41–49 (51 × 44; n

= 3), left testis 33–60 × 34–60 (50 × 45; n = 5). Cirrus sac pyriform, thin-walled, posteriorly overlapping with anterior margin of ventral sucker, 77–113 × 40–60 (94 × 50; n = 8). Cirrus sac containing coiled seminal vesicle and unarmed, muscular cirrus. Prostatic glands not observed. Genital pore sub-median, between oral and ventral suckers. Ovary not observed, probably obscured by vitellarium and uterus. Seminal receptacle, Mehlis' gland and Laurer's canal not observed. Vitellarium consists of numerous small follicles, dextral or sinistral in hinbody, principaly obscured by uterus. Uterus thin-walled, strongly developed, occupying the entire hindbody, rarely extending slightly anterior to forebody, typically overlapping gonads. Metraterm well-developed, thick-walled, strongly muscular. Eggs operculate, 28–31 × 17–20 (29 × 19; n = 8). Body length:egg length ratio 12.28–13.94 (13.31; n = 8). Excretory pore and excretory vesicle not observed.

Remarks

Anenterotrema kawsayense **n. sp.** is typified by having protuberances on the anterior margin of the oral sucker. The new species slightly resembles *A. hastati* **n. sp.** by having unicellular glands located anterolateral to the cirrus sac. However, *A. kawsayense* **n. sp.** is easily distinguished from *A. hastati* **n. sp.** by body shape (elliptoid in *A. kawsayense* **n. sp.** vs pyriform in *A. hastati* **n. sp.**), oral sucker shape (very broadly elliptoid in *A. kawsayense* **n. sp.** vs almost clamp-shaped in *A. hastati* **n. sp.**), and by the position of the testes (overlapping with ventral sucker in *A. kawsayense* **n. sp.** vs located immediately posterior at ventral sucker in *A. hastati* **n. sp.**).

Anenterotrema peruense n. sp. Cacique, Cruces & Chero

Description (Fig. 1D, Figs. 2M-N)

Type-host: *Phyllostomus hastatus* (Pallas) (Chiroptera: Phyllostomidae), greater spear-nosed bat.
Site in host: duodenum.
Prevalence: one of four hosts infected (25%) with a total 15 worms.
Mean Intensity: 15 digeneans per infected host.
Co-infection: This species was found infecting *P. hastatus* along with *A. hastati* **n. sp.**
Type-locality: Kawsay Biological Station (12°31'44''S, 69°0'26''W), Madre de Dios, Peru, South America.
Type-material: Holotype (MUSM-HEL 5126); 6 paratypes (MUSM-HEL 5127a-f).
ZooBank registration: The Life Science Identifier (LSID) for *Anenterotrema peruense* **n. sp.** is urn:lsid:zoobank.org:act:8FCDBEA6-43B4-424B-A6FF-B4210E6E6188.
Etymology. The specific name refers to the country where the species was found.

Based on 7 adult specimens. Body almost subtriangular, 262–310 (281; n = 7) long; greatest width, 240–293(265; n = 7) at level anterior of ventral sucker. Body width:length ratio 0.84–1.04 (0.94; n = 7). Forebody length 106–142 (124, n=7). Forebody: body length ratio 0.39–0.48 (0.44 = 7). Tegument smooth at anterior and posterior end, corrugated in midline of body. Oral sucker subterminal, broadly oval, 75–120 × 128–145 (95 × 137; n = 7). Oral sucker length: body length 0.27–0.39 (0.34; n = 7). Ventral sucker oval, located near posterior end of body, 93–127 × 127–146 (114 × 134; n = 7). Ventral sucker width: body width ratio 0.47–0.57 (0.51; n = 7). Oral sucker width:ventral sucker width ratio 0.97–1.10 (1.02; n = 7). Pharynx, esophagus and intestinal caeca absent. Testes opposite, oval, principally pre-equatorial, located principally anterior to ventral sucker, right testis 34–43 × 48–62 (38 × 52; n =7), left testis 37–39 × 42–59 (38 × 52; n = 4). Cirrus sac perpendicular to midline of body, thin-walled, overlapping with oral and ventral suckers, 54–78 (63; n = 7) wide. Cirrus sac containing coiled seminal vesicle and unarmed, muscular cirrus. Prostatic glands not observed. Genital pore median, between oral and ventral suckers. Ovary 33–47 × 38–52 (40 × 47; n = 3), posterior to testes, near posterior end, overlapping to ventral sucker. Seminal receptacle, Mehlis' gland and Laurer's canal not observed. Vitellarium consists of numerous small follicles, dextral or sinistral in hinbody, principaly obscured by uterus. Uterus thin-walled, strongly developed, occupying entire hindbody, extending slightly anterior to forebody, typically overlapping gonads. Metraterm not observed. Eggs operculate, 27–31 × 18–20 (29 × 18; n = 7). Body length:egg length ratio 9.03–11.07 (9.82; n = 7). Excretory pore terminal; excretory vesicle not observed.

Remarks

Anenterotrema peruense **n. sp.** can be distinguished from all other species of *Anenterotrema* by having the testes located principally anterior to ventral sucker and the cirrus sac located perpendicular to midline of the body. An *Anenterotrema* species, *A. liliputianum* (Travassos, 1928) Caballero, 1964, have been described having the testes located at ventral sucker level. However, the new species differs from *A. liliputianum* by having suckers with variably shaped aperture (suckers with spherical aperture) and by the position of the ovary (overlapping with ventral sucker in the new species vs posterolateral to ventral sucker in *A. liliputianum*). In addition, the new species further differs from *A. liliputianum* in having a smaller forebody:body length (0.39–0.48 in the new species vs 0.58–0.6 in *A. liliputianum*) and a much large body width:body length (0.84–1.04 in the new species vs 0.66–0.74 in *A. liliputianum*) and body:egg length (13.0–19.8 in the new species vs 9–13 in *A. liliputianum*).

Discussion

Four new species of *Anenterotrema* infecting two leaf-nosed bat hosts in the Neotropical region are described here, increasing to 12 the valid species in the genus (Fernandes et al., 2021a). Currently, species of *Anenterotrema* have been described infecting the small intestine, rarely the gallbladder, of Neotropical bats and are characterized by the lack of digestive system organs (Gibson & Santos, 2008; Lunaschi & Drago, 2011; Achatz et al., 2018; Fernandes et al., 2021a). The primary characters used to diagnose species of *Anenterotrema* are associated with the format of the oral and ventral sucker, position of the testes, position of ovary, and size of the eggs (Lunaschi & Drago, 2011; Fernandes et al., 2021a). In this way, the new species described herein are differentiated mainly from the other species of *Aneneterotrema* by the morphology of the oral and ventral sucker, position of testes and size of the eggs. However, the species *A. paramegacetabulum* **n. sp.** showed morphologic likeness in the shape of its ventral sucker (transversely elongated and strongly protruding on ventral surface) with *A. megacetabulum*, but differ from *A. megacetabulum* by the absence of an aperture in the ventral sucker (longitudinal aperture in *A. megacetabulum*). Perhaps, this morphological similarity of the ventral sucker of *A. paramegacetabulum* **n. sp.** and *A. megacetabulum* is indicative of the coevolutionary history of the digenean parasites and their bat host.

Peru has an extraordinary diversity of mammals (Pacheco et al., 2021), but there are few mammal hosts that have any parasitological study. To date, the digenean parasites of bats, as well as other groups of mammals, have been poorly explored (Minaya et al., 2020). Currently, four species of trematodes have been described infecting bats from Peru, *A. iannaconei* Achatz, Cardenaz-Callirgos & Tkach, 2018, *Neodiplostomum vaucheri Dubois*,1983, *Ochoterenatrema fraternum* Freitas & Ibañez, 1963, *Paralecithodendrium naviculum* (Macy,1936) (Achatz et al., 2018; Minaya et al., 2020). *Anenterotrema iannaconei* was described from the silky short-tailed bat *Carollia brevicauda* Schinz, 1821 in Huanuco region (Achatz et al., 2018). Posteriorly, *A. iannaconei* was registered infecting the duodenum of the greatg fruit-eating bat *Artibeus lituratus* Olfers, the flat-faced fruit-eating bat *Ar. planirostris* (Spix) and the common short-tailed bat *Carollia perspicillata* Linnaeus from the Junin region (Minaya et al., 2020). Minaya et al. (2020) did not mention or differentiate the characteristics and the measurements of their specimens identified as *A. iannaconei* collected from three different hosts. As noted by Achatz et al. (2018), the broad geographic distribution of some *Anenterotrema* species along with the fact that some species can infect several bat genera and families, would suggest, in these cases, that the diversity of these digeneans is underestimated. The new species described here differ from *A. iannaconei*, principally, by the morphology of the oral and ventral suckers.

In the present work, three new species of *Anenterotrema* (*A. hastati* **n. sp.**, *A. kawsayense* **n. sp.**, and *A. peruense* **n. sp.**) were described from *P. hastatus*, host of *A. eduardocaballeroi* (Santos & Gibson, 2015). *Anenterotrema eduardocaballeroi* differ from these three new species mainly by having a collar-like tegumental thickening dorsal and lateral to the oral sucker (absent in *A. hastati* **n. sp.**, *A. kawsayense* **n. sp.**, and *A. peruense* **n. sp.**).

In the present survey, four species of dicrocoellids assigned to *Anenterotrema* are described from phyllostomid bats in Peru, increasing the number of known

species of this genus, in Peruvian territory, to 5. One undescribed species of *Anenterotrema* have been also reported parasitizing the duodenum of two leaf-nosed bats in Peru (Ibañez et al., 2005), evidencing that the diversity of *Anenterotrema* species is not fully known yet in Peru and more species can be discovered in the future.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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